Recipes for Antimicrobial Wine Marinades against *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*

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ABSTRACT: We have evaluated bactericidal activities against *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* of several antimicrobial wine recipes, each consisting of red or white wine extracts of oregano leaves with added garlic juice and oregano oil. Dose-response plots were used to determine the percentage of the recipes that resulted in a 50% decrease in colony-forming units (CFU) at 60 min (BA_{50}). Studies designed to optimize antibacterial activities of the recipes demonstrated that several combinations of the naturally occurring plant-derived ingredients rapidly inactivated the above mentioned 4 foodborne pathogens. We also showed that (a) incubation temperature affected activities in the following order: 37 °C > 21 °C > 4 °C; (b) varying the initial bacterial concentrations from 10^3 to 10^4 to 10^5 CFU/well did not significantly affect BA_{50} values; (c) storage of 3 marinades up to 2 mo did not change their effectiveness against *Salmonella enterica*; and (d) polyphenolic compounds isolated by chromatography from red wine exhibited exceptional activity at nanogram levels against 2 strains of *Bacillus cereus*. These observations suggest that antimicrobial wine formulations have the potential to improve the microbiological safety of foods.

Keywords: antibacterial activities, antimicrobial wine recipes, *Bacillus cereus, Escherichia coli* O157:H7, garlic juice, *Listeria monocytogenes*, microbial food safety, oregano leaves, oregano oil, *Salmonella enterica*

Introduction

As is well known, pathogenic strains of *Bacillus cereus*, *Campylobacter jejune*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* cause foodborne illnesses (Frantamico and others 2005). In an effort to define the chemical basis for bactericidal effects of natural compounds, we had previously determined the antimicrobial effects of more than 200 plant essential oils and their active components, as well as phenolic compounds, tea catechins and theaflavins, and tea infusions against pathogenic bacteria (Friedman and others 2002, 2003, 2006b; Friedman 2007). In related studies we showed that selected compounds were also active against antibiotic-resistant bacteria (Friedman and others 2004a, 2006a; Friedman 2006) and in foods. The latter include apple juices (Friedman and others 2004b), tomato and vegetable juices (Friedman and others 2006d), edible films prepared from apples (Rojas-Graü and others 2006, 2007), wines (Friedman and others 2006c), and ground beef, chicken, pork, and turkey (Juneja and others 2006a, 2006b, 2007; Juneja and Friedman 2007). These studies provide candidates for use in antimicrobial formulations to reduce pathogens in foods.

With respect to wines, we previously examined the antimicrobial activities of several plant essential oils and oil compounds when added to Chardonnay, Pinot Noir, and Sherry wines against the foodborne pathogens *E. coli* O157:H7 and *S. enterica*. The wine solutions, at low concentrations of added plant antimicrobials, exhibited high activities against the pathogens. Related high-performance liquid chromatography (HPLC) studies showed that wines can be used to extract the antimicrobials carvacrol and thymol from readily available store-bought oregano and thyme leaves. Our studies indicated that wines appear to be effective solvents for plant-derived antimicrobial formulations. Other studies suggest that wines possess antimicrobial properties (Just and Daeschel 2003; Moretro and Daeschel 2004), and that wine marinades inhibited the formation of potentially carcinogenic heterocyclic amines in fried chicken (Busquets and others 2006).

To further improve microbial food safety, the main objective of the present study was to explore the effectiveness of wine formulations containing a mixture of active compounds against 4 pathogenic organisms: *B. cereus*, *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica*. These include wine extracts of oregano leaves (Friedman and others 2006c), oregano plant essential oil (Friedman and others 2002), and garlic juice previously shown to exhibit antimicrobial effects (Dankert and others 1979; Unal and others 2001; Kim and others 2004). The ultimate goal is to develop safe, effective, and economically viable antimicrobial marinades and dressings that inactivate foodborne pathogens on salads, meat, and poultry.

Materials and Methods

Materials

Store-bought ingredients used for marinade recipes include cold-pressed garlic juice (Garlic Valley Farms, Glendale, Calif., U.S.A.), oregano leaves (McCormick and Co. Inc., Hunt Valley, Md., U.S.A.), Beringer Pinot Noir 2003 (alcohol 13.9% by volume), and Wente Chardonnay 2002 (alcohol 13.5% by volume). Oregano oil was purchased from Lhasa Herb Co. (Berkeley, Calif., U.S.A.). "Red wine polyphenolics" (granular powder) were isolated from Kenwood Pinot Noir 2002 as described below.

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Solvents and buffers

The pH values of individual ingredients of the marinade recipes are garlic juice, 3.45; Wente Chardonnay wine, 3.40; and Beringer Pinot Noir wine, 3.70. The following diluent was used for the antimicrobial assays: saline, pH 3.5 (1.74 g NaCl/200 mL H_2O adjusted to acid pH with 1 N HCl), designated as saline. Sodium chloride was added to the garlic juice and wines (0.87 g NaCl/100 mL). Phosphate buffered saline (50 mM PBS, pH 7.0), designated as PBS, was prepared by mixing 67 mL of 100 mM dibasic sodium phosphate with 33 mL of 100 mM monobasic sodium phosphate followed by the addition of 100 mL water and 1.7 4 g NaCl.

Marinade formulation

The final marinade recipe (red wine [RW]/oregano leaves [OL]/garlic juice [GJ]/oregano oil [OO]) was formulated by addition of 4 g oregano leaves to 66 mL red or white wine/saline. The oregano leaves were allowed to soak in the wine for 1 wk, as described previously (Friedman and others 2006c). Garlic juice/saline (30 mL) and oregano oil (50 μL) were then added prior to the bactericidal assay. Oregano oil at the concentrations used was soluble in the wine formulation. Table 1 shows the composition of each marinade.

Sources of bacteria

Table 2 lists the strain designations and sources of the bacteria as well as the compositions of the bacterial suspensions evaluated in the present study.

Preparation of samples for bactericidal assays

Suspensions (100 mL) of various marinade recipes (Table 1) were shaken for 10 s. Aliquots (1 mL) of suspension were then drawn for dilutions with saline. The suspension was vortexed briefly followed by a dilution of 500 μL with 500 μL saline (1/2 dilution). This was repeated 4 additional times for a total of 6 dilutions as follows: (1) 100%, stock; (2) 50.0%; (3) 25.0%; (4) 12.5%; (5) 6.3%; and (6) 3.1% of RW/OL/GJ/OO. Microtiter plates (96-well tissue culture plates from Nalge Nunc, Rochester, N.Y., U.S.A.) were prepared prior to addition of bacterial suspensions. Saline negative control (100 μL) was added to each of 6 wells. Aliquots (100 μL) of the dilution series were added to each of 6 wells. Preparation for a typical experiment included a saline negative control (6 wells) and 3 test suspensions (18 wells).

Bactericidal assays (BA_{50})

The bactericidal assay described previously (Friedman and others 2006c) was adapted for this study. Modifications include incubation of bacteria and marinade suspensions at 4 °C, 21 °C, and 37 °C and increased initial bacterial loads in the microtiter wells, that is, 10^4 and 10^5 CFU (colony-forming units)/well. Table 2 shows the composition of bacterial suspensions used in the assays as well as the sources and original strain names.

B. cereus, *E coli*, and *S. enterica* bacteria were stored on streaked plates subcultured for 16 to 18 h at 37 °C using Luria-Bertani (LB) agar plates (Difco Laboratories, Sparks, Md., U.S.A.). *L. monocytogenes* was subcultured on brain heart infusion (BHI) agar plates (Difco Laboratories). An isolated CFU was harvested with a sterile loop and suspended in 5 mL LB or BHI broth in a 15-mL sterile plastic tube. The tubes were capped and incubated with shaking (200 rpm) for 18 h.

Bacterial suspensions were prepared for the growth of CFU amounting to about 100 to 200 CFU per lane on square LB or BHI plates used for convenient counting. LB or BHI broth cultures were vortexed briefly. Aliquots (1 mL) were then added to a 1.9 mL microfuge tube. The bacterial suspension was then pelleted in a microfuge (15800 *g*) for 1 min. The supernatant was removed and sterile PBS was added to the pellet. The pellet was suspended by gentle repeated aspiration in and out of a transfer pipette. For *E. coli* and *S. enterica*, the sample's optical density at 620 nm was adjusted to about 0.4 (250 μL bacterial suspension plus 750 μL PBS). The dilution was identical for *L. monocytogenes*, but the optical density was about 0.2. *B. cereus* suspensions were not diluted (optical density about 1.0). The suspension was then added to PBS (Table 2). The control saline suspension (5 mL) or marinade was vortexed and poured onto a sterile plastic Petri dish. Bacterial suspensions (50 μL) were pipetted with a multichannel Eppendorf pipette (Hamburg Germany) using six channels and added to 6 microtiter plates' wells, which correspond to a set of negative controls or test doses. Following 60-min incubation at 4 °C, 21 °C, or 37 °C, samples (10 μL) from each of 6 wells were drawn and 6 drops were spotted at the top of an LB (*E coli*, *S. enterica*, or *B. cereus*) or BHI (*L. monocytogenes*) agar square plate. The plate was tilted and the drops were tapped to the bottom of the plate. Each test dose set was sampled and plated 4 times for 3 test doses, resulting in 12 plates from a pellet processed from 1 species. The negative controls were sampled in duplicate onto 2 plates and the 12 streaks (10 μL) were used for the average control value in the percent kill calculation (see below). Plates were allowed to dry for 5 to 7 min before transfer to a 37 °C incubator. *E. coli*, *S. enterica*, and *L. monocytogenes* were incubated overnight. *B. cereus* was incubated for 12 h before scoring for CFU.

As shown in Table 2, further dilution was required for bacterial loads of 10^4 and 10^5 cells before the plates could be spotted.

Table 1—Marinade recipe formulations

Recipe	Saline, pH 3.5 (mL)	Wine containing 0.87% NaCl (mL)	Oregano leaves (g)	Garlic juice containing 0.87% NaCl (mL)	Oregano oil (μL)
Garlic juice,[a] pH 3.5 (A)	0	0	0	100	0
Red wine[b]/oregano leaves[c] (B)	30	66	4	0	0
Red wine/oregano leaves/garlic juice (C)	0	66	4	30	0
Red wine/oregano leaves/oregano oil (0.01%)	30	66	4	0	10
Red wine/oregano leaves/oregano oil (0.02%)	30	66	4	0	20
Red wine/oregano leaves/oregano oil (0.03%)	30	66	4	0	30
Red wine/oregano leaves/oregano oil (0.05%) (D)	30	66	4	0	50
Red wine/oregano leaves/oregano oil (0.1%)	30	66	4	0	100
Red wine/oregano leaves/garlic juice/oregano oil (0.05%) (E)	0	66	4	30	50
White wine[d]/oregano leaves/garlic juice/oregano oil (0.05%) (F)	0	66	4	30	50

[a]Garden Valley Garlic Juice – 30% in formulations.
[b]Beringer Pinot Noir 2003.
[c]McCormick Oregano Leaves – 4% in formulations.
[d]Wente Chardonnay 2002.

Table 2—Sources and strain designations of bacteria and composition of bacterial suspensions of *B. cereus*, *E. coli*, *L. monocytogenes*, and *S. enterica* used in the assays

Bacteria/ strain[a]	Original strain designation	Desired concentration in well (CFU)	Dilution after pellet suspension, bacteria + PBS pH 7 (μL)	OD	Dilution after OD determination, bacteria + PBS pH 7 (μL)	Final dilution prior to incubation, bacteria + saline pH 3.5	Dilution from well prior to application to plate, bacteria + saline pH 3.5 (μL)
B. cereus		10^3	None	1.2	40 + 960	160 μL + 5 mL	None
RM3190	RM3190[b]	10^4	None	1.2	40 + 960	1.6 mL + 3.4 mL	50 + 450
RM5141	T[c]	10^5	None	1.2	400 + 600	1.6 mL + 3.4 mL	10 + 990
E. coli		10^3	250 + 750	0.4	20 + 980	80 μL + 5 mL	None
RM 1484	SEA13B88[d]	10^4	250 + 750	0.4	20 + 980	800 μL + 4.2 mL	50 + 450
		10^5	250 + 750	0.4	200 + 800	800 μL + 4.2 mL	10 + 990
L. monocytogenes		10^3	250 + 750	0.2	20 + 980	40 μL + 5 mL	None
RM2199	F2379[e]	10^4	250 + 750	0.2	20 + 980	400 μL + 4.6 mL	50 + 450
		10^5	250 + 750	0.2	200 + 800	400 μL + 4.6 mL	10 + 990
S. enterica serovar Hadar RM1309		10^3	250 + 750	0.4	20 + 980	40 μL + 5 mL	None
	MH136[f]	10^4	250 + 750	0.4	20 + 980	400 μL + 4.6 mL	50 + 450
		10^5	250 + 750	0.4	200 + 800	400 μL + 4.6 mL	10 + 990

[a]Strain file designation (RM) of Produce Safety and Microbiology Research Unit of this laboratory.
[b]Isolated in this laboratory by Dr. Michael Cooley from soil. Identified by 2 separate generally accepted bacterial identification systems: Biolog, Hayward, Calif. and Microbial ID, Newark, Del.
[c]Bacillus Genetic Stock Center, Ohio State Univ., Columbus, Ohio.
[d]Provided by the FDA; isolated from apple juice, associated with outbreak.
[e]Provided by Univ. of California, Berkeley; isolated from cheese associated with outbreak.
[f]Provided by Dr. Lisa Gorski of this laboratory; isolated from contaminated ground turkey.

CFU were enumerated for each 10-μL streak using a colony counter.

Estimation of bactericidal activities (BA_{50} and CFU assay values)

A 2nd assay, referred to as the CFU assay, was used with *L. monocytogenes* when the BA_{50} test with 67% marinade was estimated to be greater than 67% but less than 100%. In this assay, bacterial suspensions were added to 5 mL of undiluted (100%) marinade. The 6 negative control wells were sampled twice at 0, 30, or 60 min. The decreases in CFU numbers were calculated as percent decrease compared with matched control for the 3 time points.

The numbers of CFU counted for the six 10-μL streaks from negative controls or test marinade plates were transferred to a Microsoft Excel 8.0 spreadsheet. Each sample was tested initially as a series of 6 dilutions (2.1% to 67%)). The observed CFU values were also transferred in order to determine the % CFU reduction for each well when compared to the averaged negative control value ($N = 12$), determined on 2 separate plates. Each of the dose-response profiles was examined graphically (Cricket Graph) and the BA_{50} values were estimated by linear regression. In some cases, BA_{50} values were estimated when the CFU reduction was less than 50% (the lowest value was 30%).

Isolation of wine polyphenolics

The method for polyphenol extraction was adapted from the literature (Nigdikar and others 1998). The wine (700 mL) was first de-alcoholized by evaporating off the ethanol on a Büchi roto-evaporator (Buchi Corp., New Castle, Del., U.S.A.), under vacuum, at ambient temperature. The resulting concentrate was passed through a low-pressure 2 × 15 cm column of Diaion® HP-20 resin (Supelco, Bellefonte, Pa., U.S.A.). The column was washed with 4 volumes of water. Flow rate was approximately 1 drop/2 s. Polyphenols were collected in 4 volumes of 50% ethanol. The eluent was completely dried by roto-evaporation. The residue was taken up in water and transferred to a lyophilizing flask. A small amount of residue was insoluble in water. A minimal amount of 95% ethanol was used to bring the remaining residue to solution. Care was taken to minimize the amount of ethanol used in this step because too high a concentration of ethanol causes the sample to melt prematurely during lyophilization. The ethanol extract was added to the water extract and the combined extracts were then lyophilized. Yield (about 1.8 g of dark red polyphenols from 700 mL of original wine) was determined by weighing the lyophilizing flask before and after freeze-drying.

The phenolic content of the extract was determined by reacting with Folin-Ciocalteau reagent (Sigma, St. Louis, Mo., U.S.A.) using gallic acid to determine the standard response curve, as described by Singleton and Rossi (Singleton and Rossi 1965). The powder contained 45% by weight of phenolic compounds.

Results and Discussion

Bactericidal assays

We prefer to use the BA_{50} assay to measure bactericidal activity because it can be obtained from the linear part of plots of concentration of a dilution series compared with activity. As is the case with LD_{50} values widely used in animal toxicity studies as well as with EC_{50} values we used in cancer cell inhibition studies (Friedman and others 2007), the midpoint of such plots gives the best precision of the activity. Minimum inhibitory concentration (MIC) values can be obtained from the dose-response plots (Friedman and others 2002). The easy-to-perform microtiter-based bactericidal assay facilitates

screening and comparisons of activities of large numbers of plant-derived compounds against microbial pathogens.

Antibacterial activities of marinades against *E. coli*, *Salmonella*, and *Listeria*

Based on previous findings that wine extracts of store-bought oregano leaves (Friedman and others 2006a) as well as garlic juice (Dankert and others 1979; Unal and others 2001) exhibited antimicrobial activities against foodborne pathogens, we evaluated the following combinations of these natural antimicrobials against *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica*: garlic juice (A in Table 1 and 3), a red wine extract of oregano leaves (B in Table 1 and 3), a red wine extract of oregano leaves plus garlic juice (C in Table 1 and 3), red wine extracts of oregano leaves with 5 concentrations (0.01, 0.02, 0.03, 0.05, or 0.10%) of oregano oil (D in Table 1 and 3), a red wine extract of oregano leaves plus garlic juice and 0.05% oregano oil (E in Tables 1 and 3), and a white wine extract of oregano leaves plus garlic juice and 0.05% oregano oil (F in Table 1 and 3). Table 3 shows the BA_{50} values for these samples.

At incubation temperatures of 4 °C and 21 °C, garlic juice was inactive against the 3 pathogens under the test conditions. After incubation of 37 °C, garlic juice (A) exhibited only marginal activity against *E. coli* but significant activity against *L. monocytogenes* (BA_{50} = 48.9; that is, a 48.9% solution of the marinade in saline killed 50% of bacteria after 60 min), and higher activity (lower BA_{50}) against *S. enterica* (BA_{50} = 17.1; that is, a 17.1% solution of garlic juice in saline killed 50% of the bacteria after 60 min). Table 3 also shows that the inhibitory activities of the garlic juice solutions against initial levels of *S. enterica* of 10^3, 10^4, or 10^5 CFU/well were similar, as was incubation at 120 min at 37 °C with 10^3 CFU/well.

For the red wine extract of oregano leaves (B), Table 3 shows that at incubation at 4 °C, there was no activity against *E. coli* and *Listeria*, and significant activity (BA_{50} = 43.0) against *S. enterica*. High activity was observed for all 3 pathogens after incubation at 21 °C or 37 °C. The BA_{50} values for *E. coli* ranged from 17.1 to 24.4; for *Listeria*, from 20.3 to 38.5; and for *S. enterica*, from 8.8 to 13.1.

For the red wine extract of oregano leaves plus garlic juice (C), the data show that at incubation at 4 °C, the recipe was inactive against

Table 3—Antibacterial activity (BA_{50}[a]) of marinades against *E. coli*, *L. monocytogenes*, and *S. enterica*

Recipe	Assay diluent	Assay conditions	BA_{50}[a] *E. coli* O157:H7	BA_{50}[a] *Listeria monocytogenes*	BA_{50}[a] *Salmonella enterica*
Garlic juice,[b] pH 3.5 (A)	Saline, pH 3.5	4 °C, 60 min	>67[h]	>67	>67
		21 °C, 60 min	>67	>67	>67 (82) [x]
		37 °C, 60 min, 10^3	>67 (75)[i]	48.9 ± 4.5[u]	17.1 ± 4.9[y]
		37 °C, 60 min, 10^4[f]	ND[j]	ND	10.4 ± 2.0[z]
		37 °C, 60 min, 10^5[g]	ND	ND	14.4 ± 3.1[a]
		37 °C, 120 min	>67 (78)[k]	ND	15.3 ± 2.1[n]
Red wine[c]/oregano leaves[d] (B)	Saline, pH 3.5	4 °C, 60 min	>67 (82)[l]	>67	43.0 ± 2.9[n]
		21 °C, 60 min	24.4 ± 3.1[m]	38.5 ± 1.9[n]	13.1 ± 1.4[u]
		37 °C, 60 min	20.7 ± 1.0[m]	21.0 ± 1.5[m]	11.2 ± 0.4[t]
		37 °C, 60 min, 10^4[f]	19.7 ± 2.2[m]	20.3 ± 1.5[m]	10.1 ± 0.6[t]
		37 °C, 60 min, 10^5[g]	17.1 ± 2.5[m]	26.4 ± 0.9[m]	8.8 ± 0.7[t]
Red wine/oregano leaves/garlic juice (C)	Saline, pH 3.5	4 °C, 60 min	>67	>67	48.5 ± 8.5[n]
		21 °C, 60 min	>67	46.5 ± 2.5[t]	20.0 ± 3.1[r]
		37 °C, 60 min	44.0 ± 1.8[n]	42.8 ± 3.2[t]	12.5 ± 0.6[t]
Red wine/oregano leaves/oregano oil (0.01%)	Saline, pH 3.5	4 °C, 60 min	>67 (85)[o]	>67	ND
Red wine/oregano leaves/oregano oil (0.02%)	Saline, pH 3.5	4 °C, 60 min	>67 (71)[p]	>67	ND
Red wine/oregano leaves/oregano oil (0.03%)	Saline, pH 3.5	4 °C, 60 min	>67 (69)[q]	>67 (80)[v]	ND
Red wine/oregano leaves/oregano oil (0.05%) (D)	Saline, pH 3.5	4 °C, 60 min	53.7 ± 8.2	>67 (69)[w]	ND
Red wine/oregano leaves/oregano oil (0.1%)	Saline, pH 3.5	4 °C, 60 min	47.2 ± 2.1[s]	54.1 ± 6[t]	ND
Red wine/oregano leaves/garlic juice/oregano oil (0.05%) (E)	Saline, pH 3.5	4 °C, 60 min	52.7 ± 6.0[r]	>67	22.7 ± 1.5[r]
	Saline, pH 3.5	4 °C, 60 min, 10^4[f]	44.5 ± 6.8[t]	ND	19.8 ± 2.1[t]
	Saline, pH 3.5	4 °C, 60 min, 10^5[g]	37.0 ± 6.0[t]	ND	21.1 ± 1.2[t]
	Saline, pH 3.5	21 °C, 60 min	38.5 ± 4.7[t]	53.1 ± 8.1[r]	5.8 ± 0.5[t]
	Saline, pH 3.5	37 °C, 60 min	20.5 ± 1.2[m]	20.3 ± 1.1[t]	7.3 ± 0.4[s]
White wine[e]/oregano leaves/garlic juice/oregano oil (0.05%) (F)	Saline, pH 3.5	37 °C, 60 min	18.5 ± 2.2[r]	23.4 ± 0.8[n]	5.8 ± 0.4[t]
	Saline, pH 3.5	4 °C, 60 min, 10^5[g]	37.5 ± 5.3[t]	49.3 ± 2.6[n]	19.8 ± 1.1[t]

[a] BA_{50} – percentage of marinade in well that caused 50% reduction in CFU.
[b] Garden Valley Garlic Juice – 30% in formulations.
[c] Beringer Pinot Noir 2003.
[d] McCormick Oregano Leaves – 4% in formulations.
[e] Wente Chardonnay 2002.
[f] Incubated with 104 cells in well; all incubations not showing initial load were done with 103 CFU/well.
[g] Incubated with 105 cells in well.
[h] >67: activity below 50% reduction in CFU – in the BA_{50}, 67% is the maximum concentration of marinade in well.
[i] N = 4: one BA_{50}, 3 estimated values from regression lines with < 50% CFU reduction, 4 negative values.
[j] ND: not done.
[k] N = 5: two BA_{50}s, 3 estimated values from regression lines with < 50% CFU reduction, 1 negative value.
[l] N = 1: one estimated value from regression line with < 50% CFU reduction, 3 negative values.
[m] N = 8.
[n] N = 4.
[o] N = 1: one estimated value from regression line with < 50% CFU reduction, 11 negative values.
[p] N = 6: three BA_{50}s, 3 estimated values from regression lines with < 50% CFU reduction, 6 negative values.
[q] N = 9: five BA_{50}s, 4 estimated values from regression lines with < 50% CFU reduction, 3 negative values.
[r] N = 10.
[s] N = 11.
[t] N = 12.
[u] N = 16.
[v] N = 3: three estimated values from regression lines with < 50% CFU reduction, 9 negative values.

E. coli and *Listeria*, but was active against *S. enterica* (BA_{50} = 48.5). After incubation at 21 °C, this marinade was inactive against *E. coli*, but was active against *Listeria* (BA_{50} = 46.5) and *S. enterica* (BA_{50} = 20.0). After incubation at 37 °C, this recipe exhibited activity against all 3 pathogens, with BA_{50} values against *E. coli* of 44.0; against *Listeria* of 42.8; and against *S. enterica* of 12.5.

Marinades with red wine and oregano leaves were evaluated at 4 °C with increasing amounts of oregano oil for activities against *E. coli* O157:H7 and *L. monocytogenes*. Some combinations exhibited no activity against the 2 pathogens. However, adding 0.05% of oregano oil to the recipe resulted in observed activity against *E. coli* (BA_{50} = 53.1). With added 0.10% oregano oil, activity was also observed against *L. monocytogenes* (BA_{50} = 54.6). Recipe E consisting of a red wine extract of oregano leaves plus garlic juice and 0.05% oregano oil was active against the 3 pathogens at the 3 incubation times, with the exception of *L. monocytogenes* at 4 °C. Similar high activity was observed with recipe F tested with 10^3 CFU/well at 37 °C and with 10^5 CFU/well at 4 °C.

The cited findings indicate that (a) oregano leaves, widely used in culinary practices in homes and restaurants, can provide a ready source of natural antimicrobials for the preparation of antimicrobial wine marinades; (b) activity against the 3 pathogens did not vary significantly with initial numbers of bacteria in the range of 10^3 to 10^5 CFU/well; (c) activity increased with incubation temperature in the range of 4 °C to 37 °C; (c) the color of the wine, red or white, did not affect the high activities of recipes E and F.

Storage stabilities of marinades

To ascertain the shelf life (age) of marinades with regard to their antimicrobial potency, assays were carried with 3 marinades stored at room temperature for 1 d, 1 wk, 1 mo, and 2 mo, respectively. Table 4 shows that the bactericidal properties of the 3 marinades against *S. enterica* were largely unaffected by the age of the marinades stored up to 2 mo. The red wine extract of oregano leaves, the corresponding extract with garlic juice, and the extract with garlic juice and 0.05% oregano oil were highly bactericidal against *Salmonella* cells. Activities in terms of BA_{50} value ranged from 3.7 to 12.5. These results indicate that these marinades can be stored at home or elsewhere for up to 2 mo without loss of effectiveness.

Inactivation of *L. monocytogenes* by marinades determined by the CFU assay

To establish whether the CFU assay, which measures the decrease in the number of CFU in samples without dilution with saline, can be used to evaluate antimicrobial activities in the marinades, we measured the survival of *Listeria* at 4 °C at 3 time periods. Table 5 shows that the red wine extract of oregano leaves with garlic juice and 0.05% oregano oil instantly killed the bacteria (see about 1 to 2 min column). The corresponding bactericidal effect caused by the red wine extract with garlic juice was 91% at 0 time and 100% at both 30 min and 60 min. The red wine extract of the oregano leaves killed 58% of the bacteria at 0 time and 100% at both 30 and 60 min. Red wine alone had no effect at 0 time but killed 94% of the *Salmonella* at 30 min and 100% at 60 min. These results show that the 3 marinades instantly killed *Listeria*, but that the red wine alone required approximately 30 min to kill all bacteria.

Inactivation of *B. cereus* by garlic juice, marinades, wines, and wine polyphenolics

Previously, we found that tea flavonoids (polyphenolic compounds) exhibited exceptionally high activity at nanomolar levels against the *B. cereus* strain RM3190 (Friedman and others 2006b). It was therefore of interest to find out whether a different source of phenolic compounds, those present in wines, would also exhibit high activities against *B. cereus*. Table 6 summarizes our finding with 2 strains of *B. cereus*. The data show that garlic juice in saline, 2 marinades consisting of red or white wine extracts of oregano leaves plus garlic juice and 0.05% oregano oil, red and white wines, and the isolated red wine polyphenolics were highly active against both pathogenic strains. Moreover, the red wine polyphenolics exhibited exceptionally high activity at nanogram levels (BA_{50} = 0.000059%),

Table 4—Effect of marinade shelf life on bactericidal activity (BA_{50}[a]) against *S. enterica*

Recipe	Conditions	BA_{50}[a] 1 D	1 wk	1 mo	2 mo
Red wine[b]/oregano leaves[c]	37 °C, 60 min	11.2 ± 0.4[e]	11.8 ± 0.6[g]	11.1 ± 0.4[e]	8.3 ± 1.5[e]
Red wine/oregano leaves/garlic juice[d]	37 °C, 60 min	12.5 ± 0.6[e]	10.1 ± 1.1[e]	6.3 ± 0.6[g]	8.6 ± 1.3[e]
Red wine/oregano leaves/garlic juice/oregano oil (0.05%)	37 °C, 60 min	5.3 ± 0.4[f]	7.3 ± 1.4[e]	5.4 ± 0.3[e]	3.7 ± 0.5[f]

[a] BA_{50} – percent marinade in microtiter well that caused 50% reduction in CFU.
[b] Beringer Pinot Noir 2003.
[c] McCormick Oregano Leaves.
[d] Garlic Valley Garlic Juice.
[e] $N = 12$.
[f] $N = 11$.
[g] $N = 8$.

Table 5—Antibacterial activity of 100% marinades against *Listeria monocytogenes* at 4 °C incubation determined by the CFU assay

Recipe	Assay conditions	Percent difference in CFU compared with time-matched control[a] About 1–2 min	30 min	60 min
Red wine[b]/oregano leaves[c]/garlic juice[d]/oregano oil (0.05%)	4 °C, pH 3.5	–100 ± 1[e]	–100 ± 0	–100 ± 0
Red wine/oregano leaves/garlic juice	4 °C, pH 3.5	–91 ± 8	–100 ± 0	–100 ± 0
Red wine/oregano leaves	4 °C, pH 3.5	–58 ± 3	–100 ± 0	–100 ± 0
Red wine	4 °C, pH 3.5	6 ± 6	–94 ± 3	–100 ± 1

[a] $N = 12$; average CFU values from 12 saline, pH 3.5, control streaks were compared with average CFU values from 12 marinade streaks.
[b] Beringer Pinot Noir 2003.
[c] McCormick Oregano Leaves.
[d] Garlic Valley Garlic Juice.
[e] 100% of the bacteria were destroyed.

Table 6—Antibacterial activity (BA_{50}[a]) of marinade against 2 strains of *B. cereus*

Recipe	Assay diluent	Assay conditions	BA_{50}[a] *B. cereus* RM3190	BA_{50}[a] *B. cereus* RM5141
Garlic juice,[b] pH 3.5	Saline, pH 3.5	4 °C, 60 min	2.8 ± 0.2[h]	2.1 ± 0.4[o]
	Saline, pH 3.5	21 °C, 60 min	1.1 ± 0.4[i]	0.63 ± 0.08[p]
	Saline, pH 3.5	21 °C, 60 min, 10^4[f]	0.84 ± 0.08[j]	0.93 ± 0.15[j]
	Saline, pH 3.5	21 °C, 60 min, 10^5[g]	>8.4 (11)[k]	5.1 ± 0.9[m]
Red wine[c]/oregano leaves[d]/garlic juice/oregano oil (0.05%)	Saline, pH 3.5	4 °C, 60 min	10 ± 1.3[j]	9.5 ± 1.4[j]
	Saline, pH 3.5	4 °C, 60 min, 10^4[f]	10 ± 0.8[h]	6.5 ± 0.8[q]
	Saline, pH 3.5	4 °C, 60 min, 10^5[g]	12 ± 0.8[l]	10.7 ± 1.3[l]
	Saline, pH 3.5	21 °C, 60 min	0.66 ± 0.05[h]	0.65 ± 0.03[p]
White wine[e]/oregano leaves[d]/garlic juice/oregano oil (0.05%)	Saline, pH 3.5	21 °C, 60 min	0.26 ± 0.04[m]	0.42 ± 0.02[m]
Red wine[c]	Saline, pH 3.5	21 °C, 60 min	0.046 ± 0.015[h]	0.046 ± 0.013[o]
White wine[e]	Saline, pH 3.5	21 °C, 60 min	3.7 ± 0.1[m]	3.4 ± 1.3[m]
Beringer Pinot Noir 2002	Saline, pH 3.7	21 °C, 60 min	0.013 ± 0.003[n]	ND[r]
Kenwood Pinot Noir 2002	Saline, pH 3.7	21 °C, 60 min	0.017 ± 0.003[n]	ND
Red wine polyphenolics	Saline, pH 3.7	21 °C, 60 min	0.000059 ± 0.00002[n]	ND

[a] BA_{50} – percentage of marinade components in well that caused 50% reduction in CFU.
[b] Garden Valley Garlic Juice – 30% in formulations.
[c] Beringer Pinot Noir 2003.
[d] McCormick Oregano Leaves – 4% in formulations.
[e] Wente Chardonnay 2002.
[f] Incubated with 10^4 cells in well.
[g] Incubated with 10^5 cells in well.
[h] $N = 8$.
[i] $N = 14$.
[j] $N = 12$.
[k] $N = 3$: three estimated values from regression lines with <50% CFU reduction, one negative value.
[l] $N = 7$.
[m] $N = 4$.
[n] $N = 10$.
[o] $N = 6$.
[p] $N = 5$.
[q] $N = 9$.
[r] ND = not done.

that red wine alone was about 8 times more active than the white wine, and that the activity of the red wine marinade at an incubation temperature of 21 °C was 10 to 16 times greater than at an incubation time of 4 °C. These observations show that the wine marinades and their constituents can also be used to inactivate pathogenic *B. cereus* strains. These results reinforce our previous suggestion that phenolic compounds contribute significantly to antimicrobial activities of wines (Friedman and others 2006c).

Conclusions

In the present and previous studies, we have reported our findings on the antibacterial activities of wine extracts of oregano leaves containing the antimicrobials carvacrol and thymol, wines, wine polyphenolic compounds, garlic juice, oregano oil, and combinations of these components on foodborne human pathogenic bacteria. The results show that wines appear to be useful solvents for plant-derived antimicrobial formulations.

We have developed antimicrobial wine recipes consisting of wine extracts of oregano leaves, oregano oil, and garlic juice that exhibited strong activities under laboratory conditions; that is, they acted against 4 major foodborne pathogens. Although wine constituents including phenolic compounds, ethanol, and sulfur dioxide (Tompkin and others 1980; DiPersio and others 2003; du Toit and others 2005) undoubtedly contributed to the observed activities, activities were significantly enhanced by the presence of oregano oil. The present study further extends our knowledge about the potential use of botanicals as biopreservatives in foods (Draughton 2004; Davidson and others 2005).

Further studies should investigate their effectiveness, sensory compatibility, and safety in various applications. These include suggested uses as antimicrobial marinades for meat and poultry products (McKenna and others 2003; Sánchez-Plata and others 2005; Yoon and others 2005; Lawson 2006; Tapsell and others 2006), salad dressings, and rinses and sprays for contaminated surfaces of fruits, vegetables, meat, poultry, and nonfood items such as meat cutting boards. Consumption of feeds and foods treated with antimicrobial wine formulations may also benefit therapy of infectious diseases of animals and humans.

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